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Multiplexed *In Situ* Hybridization Using Hybridization Chain Reaction

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CHOI *ET AL.*[1] DESCRIBE PROGRAMMABLE *IN SITU* AMPLIFIERS based on the mechanism of hybridization chain reaction (HCR) that enable simultaneous mapping of multiple target mRNAs within whole-mount zebrafish embryos. In contrast to traditional *in situ* hybridization approaches, HCR *in situ* amplification is performed for all target mRNAs simultaneously, so the experimental timeline is independent of the number of target mRNAs. Using HCR, probes complementary to mRNA targets trigger chain reactions in which metastable fluorophore-labeled hairpins self-assemble into tethered fluorescent amplification polymers *in situ* (Fig. 1), enabling straightforward multiplexing for up to five target mRNAs at a time, deep sample penetration, high signal-to-background, and subcellular signal localization. The new DNA HCR technology[1] improves on the original RNA HCR technology[2] to dramatically increase signal gain, reduce reagent cost, and increase reagent durability. DNA HCR kits containing probe sets, amplifiers, and buffers can be purchased from the nonprofit academic resource (www.molecularinstruments.org). Advice and technical support can be obtained by emailing support@molecularinstruments.org

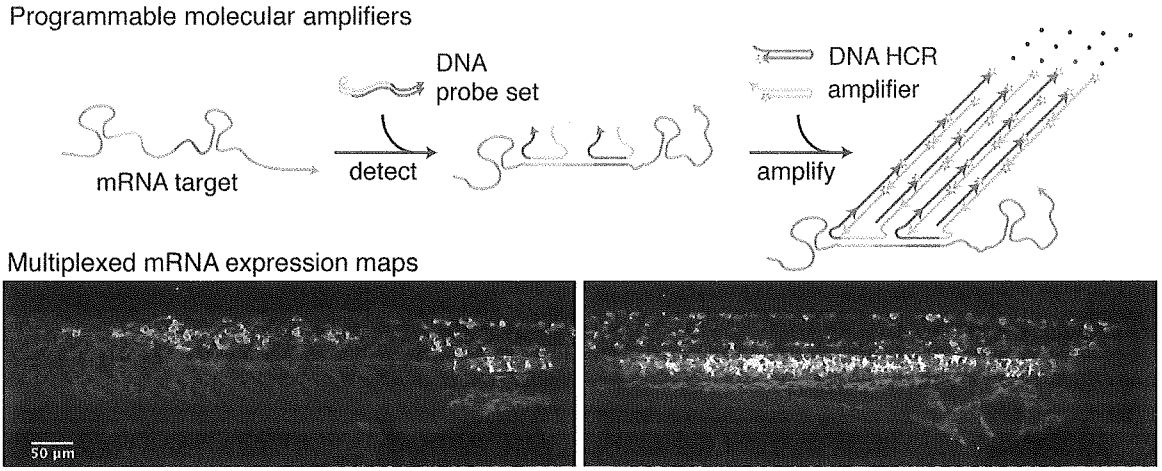

FIG. 1. Multiplexed mapping of mRNA expression within intact zebrafish embryos using next-generation DNA hybridization chain reaction (HCR). [Image from Choi *et al.* (2014); http://pubs.acs.org/doi/abs/10.1021/nn405717p; used with permission from the American Chemical Society.] Color image is available online at www.liebertpub.com/zeb

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Cover Art: Pan-cytokeratin positive cells arising from zebrafish embryonic stem cells. The pluripotent embryonic stem cells were maintained in a feeder free culture system.

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